Supplement 1: Complete formulation of optimization problem

In Eq. 4 in the main manuscript, the optimization problem was introduced in a general form. For the NET analysis of the specific set of metabolome data, the optimization was adjusted according to the following points:

- For some isobaric metabolites only lumped concentrations were available. Consequently, in the calculation the sum of the lumped metabolite concentrations, $c_l$, had to equal the measured overall concentration.

- The measured concentrations are cell-wide averages for *S. cerevisiae*. Hence, measured values equal the sum over the intracompartmental concentrations weighted by the respective volume fraction $v_m$.

- The adenylate energy charge (AEC),

$$AEC = \frac{c_{ATP} + 0.5 c_{ADP}}{c_{ATP} + c_{ADP} + c_{AMP}}$$

was restricted to physiological values derived from the literature survey (see Supplement 2). For the pyridine nucleotides, ratios between the reduced and the oxidized form were employed in the optimization and restricted to literature value ranges.

- To linearize the equations for the Gibbs energies of formation, concentrations were employed in logarithmic form.

- The standard transformed Gibbs energies of formation were allowed to vary by 0.5 kJ mol$^{-1}$ to account for errors of 20% in the determination of equilibrium concentrations from which they were derived.

The following equations display the complete formulation of the optimization problem used for the NET analysis of the *E. coli* (Eq. 1) and *S. cerevisiae* (Eq. 2) in context of this paper. Here, also the possibility to calculate the feasible range of a particular concentration $c_n$ is considered.
\[
\begin{align*}
\min / \max & \quad \ln(c_i)/\Delta r_{G_k}' \\
\text{s.t.} & \quad \Delta r_{G_j}' \leq 0 \quad \forall \quad r_j > 0 \\
& \quad \Delta r_{G_j}' \geq 0 \quad \forall \quad r_j < 0 \\
& \quad \Delta r_{G_j}' = \sum_i s_{ij}\Delta f_{G_i}' \\
& \quad \Delta f_{G_i}' = \Delta f_{G_i}^{0} + RT\ln(c_i) \\
& \quad \frac{c_{ATP} + 0.5\ c_{ADP}}{c_{ATP} + c_{ADP} + c_{AMP}} = AEC \\
& \quad \sum_i c_i = c_{\text{measured sum}} \quad \forall \quad \text{pooled metabolites} \\
& \quad \ln(c_i^{\text{min}}) \leq \ln(c_i) \leq \ln(c_i^{\text{max}}) \\
& \quad \Delta f_{G_i}^{0} - 0.5 \frac{kJ}{mol} \leq \Delta f_{G_i}^{0} \leq \Delta f_{G_i}^{0} + 0.5 \frac{kJ}{mol} \\
& \quad 0.75 \leq AEC \leq 0.95 \\
& \quad 0.005 \leq \frac{c_{NADH}}{c_{\text{NAD}^+}} \leq 0.2 \\
& \quad 0.1 \leq \frac{c_{\text{NADPH}}}{c_{\text{NAPD}^+}} \leq 10
\end{align*}
\]
\[ \min / \max \quad \ln(c_i) / \Delta_r G_r' \]
\[ \text{s.t.} \quad \Delta_r G_r' \leq 0 \quad \forall \quad r_j > 0 \]
\[ \Delta_r G_r' \geq 0 \quad \forall \quad r_j < 0 \]
\[ \Delta_r G_r' = \sum_i s_{ij} \Delta_f G_i' \]
\[ \Delta_f G_i' = \Delta_f G_i'^0 + RT \ln(c_i) \]
\[ c_{ATP_m} + 0.5 c_{ADP_m} = AEC_m \quad \forall \quad m \in \{ \text{cytosol, mitochondria} \} \]
\[ \sum_m v_m \cdot c_m = c_{\text{measured average}} \quad \forall \quad \text{non-pooled metabolites} \]
\[ \sum_l \sum_m v_m \cdot c_{l,m} = c_{\text{measured sum}} \quad \forall \quad \text{pooled metabolites} \]
\[ \ln(c_i^{\text{min}}) \leq \ln(c_i) \leq \ln(c_i^{\text{max}}) \]
\[ \Delta_f G_i'^0 - 0.5 \frac{kJ}{mol} \leq \Delta_f G_i'^0 \leq \Delta_f G_i'^0 + 0.5 \frac{kJ}{mol} \]
\[ 0.8 \leq AEC_{\text{cytosol}} \leq 0.9 \]
\[ 0.4 \leq AEC_{\text{mitochondria}} \leq 0.7 \]
\[ 0.001 \leq \frac{c_{NADH}}{c_{NAD^+ \text{ cytosol}}} \leq 0.01 \]
\[ 0.1 \leq \frac{c_{NADH}}{c_{NAD^+ \text{ mitochondria}}} \leq 1 \]
\[ 0.1 \leq \frac{c_{NADPH}}{c_{NADP^+ \text{ cytosol}}} \leq 10 \]
\[ 0.1 \leq \frac{c_{NADPH}}{c_{NADP^+ \text{ mitochondria}}} \leq 10 \]